High Mobility Group Box 1 in Children with Acute Encephalopathy and Other Convulsive Diseases

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Received date: August 24, 2017; Accepted date: September 18, 2017; Published date: September 25, 2017

Abstract

HMGB1 is known as a representative of danger-associated molecular patterns (DAMPs), which play an important role in triggering inflammatory responses. In order to know the role of high mobility group box 1 (HMGB1) in children with acute encephalopathy and other neurological disorders we analyzed HMGB1 and other cytokines in serum and cerebrospinal fluid. Comparing HMGB1 in Kawasaki disease as a control, serum levels in influenza-associated encephalopathy and West syndrome were significantly lower. However, two cases of influenza-associated encephalopathy having sequelae showed extremely high levels. The levels of HMGB1 in acute encephalopathy and purulent and aseptic meningitis in cerebrospinal fluid were low; however, they were significantly higher than those in West syndrome. Serum HMGB1 levels were correlated with those of interleukin-6 and interleukin-8 in cerebrospinal fluid. HMGB1 is suspected to have a pivotal role in pathophysiology of acute encephalopathy and other neurological disorders.

Keywords: Acute encephalopathy; West syndrome; Influenza; VEGF; IL-6

Introduction

Approximately 1000 children are affected with encephalitis/encephalopathy every year in Japan. Influenza virus is the most frequent cause at approximately one fourth [1-3]. Large amounts of inflammatory cytokines, such as interleukin (IL)-6 and tumor necrosis factor alpha (TNF-α), are produced in influenza-associated encephalopathy [1,4]. High mobility group box 1 (HMGB1) release occurs actively after cytokine stimulation as well as passively during cell death [5]. HMGB1 is known as a representative of danger-associated molecular patterns (DAMPs), which play an important role in triggering inflammatory responses [6,7]. Recently, anti-HMGB1 antibody has been reported to reduce impairment by cerebral infarction in mice [8]. To clinically investigate the role of HMGB1 in encephalopathy in children, we assayed serum and cerebrospinal fluid (CSF) levels of HMGB1 and other cytokines in encephalopathy and other convulsive diseases.

Materials and Method

Patients

Twelve children with influenza-associated encephalopathy (1 to 9 years old) and 4 patients with febrile convulsions during influenza infection were enrolled. Five children with influenza-associated encephalopathy showed serious sequelae and one child died. Six patients with West syndrome as controls with uncontrolled convulsions and 12 patients with Kawasaki disease as controls with high DAMPs were also included. CSF samples were obtained from 4 patients with acute encephalopathy (2 influenza-associated encephalopathy, one rotavirus-associated encephalopathy, and one Dravet syndrome), 9 patients with epilepsy (West syndrome and neonatal epilepsy), and 7 patients with meningitis (5 purulent and 2 aseptic). Twenty-four serum samples, which were obtained from healthy controls or those without infectious diseases, were also included as controls. All samples were obtained in the acute phase and frozen at -80°C until the assay.

Measurement of HMGB1

The concentrations of HMGB1 in serum and CSF were determined using an HMGB1 ELISA kit (Shino-test Corporation, Japan). The minimal detection limit is 2.5 ng/mL. The reproducibility is within 10%. We assayed 27 cytokines in CSF (IL-1β, IL-1RA, IL-2, IL-4, IL-5, IL-6, IL-7, IL-8, IL-9, IL-10, IL-12, IL-13, IL-15, IL-17, Exotaxin, FGF basic, G-CSF, GM-CSF, IFN-γ, IP-10, MCP-1, MIP-1β, RANTES, TNF-α and VEGF) by the Bio-Plex suspension array system (Bio-Rad Laboratories, Tokyo, Japan) and the 27 Plex Panel (Bio-Rad Laboratories, Tokyo, Japan). The minimal detection limits of each cytokine are within 0.01 to 100 pg/mL (minimum: IL-4, IL-5, IL-7, IL-13, MIP-1β; maximum: RANTES).

Statistic analysis

Statistical evaluation of the collected data was conducted using the chi-square test and Spearman’s correlation coefficient by rank test for independence for significant differences.

Results

Serum HMGB1 levels in patients with influenza-associated encephalopathy and Kawasaki diseases were statistically higher than those of patients with West syndrome (Figure 1). Serum HMGB1 levels in patients with influenza-associated encephalopathy were statistically lower than those of patients with Kawasaki disease (vasculitis) as a control. However, there were two cases with extremely high levels (>100 ng/mL) of serum HMGB1 in the group of influenza-associated
encephalopathy, similar to those in Kawasaki disease. Serum HMGB1 concentrations in controls all showed less than 81 (mean and standard deviation were 24.7 and 28.7) ng/mL. There was no statistical difference between children with influenza-associated encephalopathy with or without sequelae and febrile seizure (Figure 2). Serum HMGB1 levels in children (n=7) with West syndrome were low (5.5 ± 5.43 ng/mL) except during infection.

**Figure 1:** Serum HMGB1 levels in influenza-associated encephalopathy, Kawasaki disease, and West syndrome. There were two cases with high levels of serum HMGB1 in the group with influenza-associated encephalopathy. However, there was no statistical difference in children with West syndrome. HMGB1 levels in CSF obtained from children with acute encephalopathy (two cases with influenza-associated encephalopathy, one rotavirus-associated encephalopathy, West syndrome, and purulent meningitis) are shown in Figure 3. The CSF HMGB1 levels in acute encephalopathy and purulent meningitis were significantly higher than those in West syndrome.

Serum HMGB1 levels were statistically correlated with only IL-6 and IL-8 in CSF fluid samples obtained from patients with several diseases (Figure 4). There was no significantly correlation between serum HMGB1 and several other cytokines.

**Discussion**

In systemic vasculitis, including Kawasaki disease, serum HMGB1 levels are higher in patients with active disease compared to healthy controls and the levels are associated with disease severity and mortality. HMGB1 has been suggested to be involved in the pathogenesis of vascular diseases such as systemic vasculitis [9-11]. HMGB1 protein is a late mediator of inflammation or sepsis [12]. However, there are few reports regarding the serum and CSF levels of HMGB1 in children with neurological disorders. Ito et al. reported CSF levels of HMGB1 were not associated with acute encephalopathy due to influenza H1N1 pdm09 [13]. Momonaka et al. measured serum and CSF levels of HMGB1 in patients with influenza-associated encephalopathy and reported that serum HMGB1 levels were significantly higher in patients with poor outcomes compared to those without neurological sequelae. In contrast, there were no differences in CSF HMGB1 levels among all groups [14].

**Figure 2:** Comparison of serum HMGB1 levels in children with influenza infection is shown. There was no statistical difference in children with influenza-associated encephalopathy with or without sequelae and febrile seizures. All samples were obtained from patients with influenza-associated encephalopathy in the acute phase.

**Figure 3:** HMGB1 in cerebrospinal fluid obtained from children with acute encephalopathy (two cases with influenza-associated encephalopathy, one rotavirus encephalopathy and Dravet syndrome), West syndrome, and meningitis. The levels in acute encephalopathy and meningitis were significantly higher than those in West syndrome.

In this study, serum levels in influenza-associated encephalopathy were significantly lower compared with the levels in Kawasaki disease.
(vasculitis) as control, but higher than those in West syndrome (noninfectious diseases). However, two cases with influenza-associated encephalopathy having sequelae also showed high levels. There is no statistical significant between all groups infected influenza. Influenza virus might induce production of HMGB-1 during infection in some cases. On the other hand, the CSF levels of HMGB1 in acute encephalopathy and purulent and aseptic meningitis were higher than those in West syndrome. Serum HMGB1 levels were significantly correlated with those of IL-6 and IL-8 in CSF. These results show that HMGB1 is suspected to have a pivotal role in pathophysiology of infective acute encephalopathy focused around the neurovascular unit without a direct effect in the brain.

Conclusion
Therefore, proper diagnosis of the patients with encephalopathy and early treatment against DAMPs, including HMGB1, might improve sequelae of infective encephalopathy.

Acknowledgement
This study was supported by a grant from the Ministry of Health, Labour, and Welfare of Japan.

Conflict of Interest
The authors declare no conflicts of interest.

Ethical Considerations
The authors declare that all experiments were carried out in compliance with relevant laws and guidelines in accordance with the ethical standards of the Declaration of Helsinki.

References